

SYMPOSIUM ON ENTEROBACTERIACEAE OF MEDICAL SIGNIFICANCE: ISOLATION, IDENTIFICATION, AND PATHOGENIC POTENTIALITIES¹

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Recent and significant advances in the field of enterobacteriaceae and their medical significance made this symposium a timely one. Dr. Hardy discussed the current importance of enteric infections as medical and public health problems. He pointed out that that mortality data underestimate the magnitude of the problem, and yet they are the best index available of variations in incidence. Data for the countries of the Americas show that mortality rates are comparatively low only in the United States and Canada, being as low as two deaths per 100,000 population in some states and provinces. To the south, in 3 countries there are between 20 and 50 recorded deaths from diarrheal diseases per 100,000 population, in 4 between 50 and 100, in 6 between 100 and 200, in 1 between 200 and 300, and in 2 over 300, exceeding 1,000 in some areas. In 9 of the 16 countries diarrheal diseases are the major cause of death. Dr. Hardy emphasized the urgent need for additional knowledge on these diseases, which continue to be the chief cause of death in most areas of the world.

The problem of salmonellae and salmonellosis was surveyed by Dr. Shaughnessy. He pointed out that there are now approximately 350 serotypes, which are widely distributed in the animal kingdom. Among the types most commonly isolated from man in the United States are *Salmonella typhimurium*, *S. newport*, *S. oranienburg*, *S. montevideo*, *S. bareilly*, *S. anatum*, and *S. panama*. Isolation methods used vary from laboratory to laboratory. In Dr. Shaughnessy's ex-

perience the best single medium is the Hajna-Perry modification of the original Wilson and Blair bismuth and sulfite-brilliant green medium, and the best combination of media is selenite broth and SS agar. The methodology of serotyping has been simplified to an extent that any competent laboratory can type 98 per cent of human isolates. Phage typing has been used for three purposes: to identify a strain as a salmonella (0-1 phage); to separate the salmonellae into groups, and to separate salmonella serotypes, such as *S. typhosa*, into phagetypes as an aid to epidemiologic investigation.

Paracolobactrum was competently discussed by Dr. Stuart. He defined these microorganisms as enteric bacteria fermenting lactose and/or sucrose slowly with the production of acid or acid and gas. An infrequent culture fails to attack one or the other or both of these carbohydrates. Most *Paracolobactrum* cultures can be isolated on SS agar but an occasional strain requires eosin methylene blue agar or other comparable media. *P. coliforme* and *P. aerogenoides* can be easily distinguished from *Salmonella* in 24 hr, since the former produces indole and the latter acetylmethyl carbinol. *Paracolobactrum intermedium* can be differentiated from *Salmonella* only after lactose or sucrose fermentation, which sometimes requires several weeks, or by antigenic analysis.

Evidence for the pathogenicity of the *Paracolobactrum* group rests almost entirely on epidemiologic findings. Under conditions not yet known, at least four bioserotypes appear to be pathogenic: the "Arizona" and "Bethesda" groups—*P. intermedium*; *P. aerogenoides* (type 32011); and the "Providence" group—*P. coliforme* (type 29911). Epidemic strains of the last two types have been received by Dr. Stuart from four continents and had been tentatively classified as new types of *Salmonella* or *Shigella* or as unknown enteric organisms. Nevertheless, these four pathogenic types of *Paracolobactrum* are occasionally encountered in the stools of normal

¹ This symposium was held at the Fifty-sixth General Meeting of the Society of American Bacteriologists at Houston, Texas, on May 1, 1956. S. Marcus was the organizer; participants were: A. V. Hardy, *Florida State Board of Health, Jacksonville, Florida*; H. J. Shaughnessy, *Illinois Department of Public Health, Chicago, Illinois*; C. A. Stuart, *Brown University, Providence, Rhode Island*; R. B. Lindberg, *Army Medical Service Graduate School, Washington, D. C.*; W. E. Wheeler, *Children's Hospital and Ohio State University, Columbus, Ohio*.

individuals, as is also the case with the more pathogenic *Salmonella* and *Shigella* types.

Dr. Lindberg pointed out that the true incidence of shigellosis is not known, particularly in areas less advanced in public health practices than this country. Military experience in recent wars, including the Korean conflict, has emphasized the massive scale of bacillary dysentery. Adequate collection of specimens is basic to isolation of shigellae; although the rectal swab continues to be useful as a survey tool, passed stool specimens are not infrequently superior. He emphasized the need for adequate holding and transport media. He discussed the recently described media of Damon and Hajna. Recognition of shigellae continues to be facilitated by fermentation patterns. At the 406th Medical General Laboratory, during the Korean war, a small volume of lactose-sucrose broth as a screening and enrichment fluid was used to handle large numbers of isolates. Pour plates from negative lactose-sucrose broth tubes were prepared in a phenol-red base with ferric and thiosulfate ions present. Carbohydrates, applied in impregnated discs, permitted fermentation patterns to be recognized within 6 to 8 hr. He emphasized that serologic identification remains essential. Monovalent serotypes, including *Shigella dysenteriae*, *S. boydii*, and *S. sonnei*, are readily identified. The *S. flexneri* strains are the most complex serologically. Phase variation is a natural occurrence in *S. flexneri* types 1, 2, 3, and 4. In some type 4 outbreaks 50 per cent of strains show dissociation on primary isolation, and type specificity may disappear completely in a short time. Nine group factors are currently recognized, and four subtypes of *S. flexneri* 4, in addition to 4b, were recognized in the Far East during 1950-1953. Dr. Lindberg then emphasized the occurrence of cross-reactions between shigellae, the AD group, and *Escherichia coli*. He emphasized that thermolabile K antigens are very common in shigellae. He indicated that little precise information is available on the pathogenesis of shigellosis. The endotoxins of shigellae are similar in structure to those of *E. coli*. The neurotoxin of *S. dysenteriae* 1 has been

extensively studied, but a similar toxin does not occur in other types. A potent mucinase present in many *S. flexneri* 2a strains is absent in other serotypes and therefore does not readily explain the pathogenesis of the disease. Feeding studies in human volunteers have shown that very large doses of bacteria are required to initiate infection. Dr. Lindberg emphasized the need for further studies.

The topic of enteropathogenic *E. coli* was discussed by Dr. Wheeler. He pointed out that diarrheal diseases caused by these serogroups differ in a number of respects from salmonellosis and shigellosis. Young infants are affected almost exclusively, although a rare newborn baby may escape the clinical manifestations even though the microorganism is present in the feces. The disease is highly contagious among newborn populations, and epidemics vary considerably in severity. The disease is very prone to be associated with clinical relapses. The reasons for the high contagiousness of the disease are the great susceptibility of newborn infants and the large number of the pathogens discharged from the bowel by infected patients. Dr. Wheeler pointed out that the rate of acquisition of specific hemagglutinins against several serotypes of enteropathogenic *E. coli* is similar to the rate of acquisition of poliomyelitis virus neutralizing antibodies in tropical populations and may be taken to indicate a widespread exposure to antigen in the first years of life. However, the seemingly limited prevalence of these microorganisms among adults makes it unlikely that these antibodies result from subclinical infection. The presence of antibodies in low titer does not seem to protect against infection in every instance. There is some evidence from feeding studies in adults that one attack may be followed by protection against clinical disease upon subsequent challenge but not against reimplantation of organisms in the intestinal tract. Dr. Wheeler emphasized that medical laboratory directors should be aware of the fact that these strains can be identified only serologically and that sera for the four most common serotypes, 026, 055, 0111, and 0127, recently have become commercially available.